LOSS AND GAIN OF HEAT-TOLERANCE BY THE CRAYFISH

W. A. SPOOR

Department of Zoology, University of Cincinnati, and Stone Institute of Hydrobiology, The Ohio State University

It has been well established that a fish becomes increasingly tolerant of heat as it becomes acclimatized to higher temperatures within the range of thermal tolerance of its species, and that its acclimatization to low temperature entails a loss of heat-tolerance (Doudoroff, 1942; Brett, 1944, 1946). Inasmuch as aquatic arthropods are exposed to the same fluctuations in environmental temperature as fish, one might reasonably expect to find that they, too, are capable of gaining and losing heat-tolerance. The matter has not been explored as thoroughly for aquatic arthropods as for fish, however, and there are but few instances in which it is clear that individual animals have gained or lost heat-tolerance, *i.e.*, that they have undergone "physiological" acclimatization, as the term is used by Prosser *et al.* (1950). It is true that aquatic arthropods from warm waters have been found to be more heat tolerant than related species, or even members of the same species, inhabiting cooler waters (Mayer, 1914; Huntsman and Sparks, 1924; Fox and Wingfield, 1937; Mason, 1939; Whitney, 1939; Park, 1945; Walshe, 1948; Marlier, 1949; Bovbjerg, 1952), but in most cases the differences in tolerance can be attributed as well to selection as to physiological acclimatization (Fox, 1939).

With one possible exception, the literature bearing directly upon the problem of physiological acclimatization among aquatic arthropods indicates that they do become increasingly tolerant of heat as their environmental temperatures are increased. Huntsman (1924) showed that lobster larvae raised at temperatures between 20 and 25° C. were more heat resistant than those raised at 15°, Edwards and Irving (1943) reported the thermal death point of the sand crab, *Emerita talpoida,* to be about 10° higher in summer than in winter, and Marlier (1949) found indications that the lethal temperature of larvae of the caddis fly, *Hydropsyche angustipennis,* increased from 31° in the spring to 32° in early summer. Furthermore, Bovbjerg (1952) observed that two species of crayfish, *Orconectes propinquus* and *Cambarus fodiens*, became increasingly tolerant of temperatures between 34 and 35° as the advancing season warmed their habitats, or after they had been maintained in warm water in the laboratory for five or six weeks. The possible exception was reported by Whitney (1939), who found nymphs of the mayfly, *Baetis rhodani,* to be no more heat tolerant after 40 hours at 15° than controls maintained between 10 and 11°. In view of Brett's (1946) experience with the goldfish, however, in which it was shown that the development of an increased heat-tolerance required a latent period of from one to seven days, the latent period being longer the lower the acclimatization temperature, it may be that the absence of acclimatization in *Baetis* was more apparent than real. It seems quite possible that more than 40 hours of acclimatization were required to increase the heat-tolerance enough to be detected by the method employed.

Less seems to be known about the loss of heat-tolerance by aquatic arthropods. Aside from the foregoing observations on the sand crab and crayfish, in which the seasonal gain of tolerance suggests a previous seasonal loss, no literature bearing directly upon this matter has been found.

Relatively little appears to have been published about the temperature relations of the crayfish. The mere fact of its occurrence in the northern part of the United States shows, of course, that it can survive over a range of temperatures extending from about 0 to more than 30°, and direct observations by several authors bear this out. Crayfish are active at temperatures between 4 and 6° (Van Deventer, 1937) and under two inches of ice (Bovbjerg, 1952). They will live at 3° in the laboratory (Kyer, 1942), and adult females have been found to survive freezing overnight (Langlois, 1937). Bovbjerg (1952) has recorded that they occur in ponds that reach 30°, and that acclimatized crayfish have remained alive after a period of seven days at 34 to 35° in the laboratory. In connection with the present study it was observed that adult crayfish were abundant in a shallow river of which the surface temperature at any rate reached 32°. In view of its convenient size, longevity, availability and wide range of thermal tolerance, the temperature relations of the crayfish are of considerable interest not only to the ecologist but also to the physiologist concerned with the problem of explaining the mechanism of acclimatization to temperature. The experiments to be described in this paper were undertaken to provide further information on the thermal relations of the crayfish, especially in connection with the loss and gain of heat-tolerance.

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Material and Methods

The crayfish, *Orconectes rusticus* (Girard), were collected in July of 1953 and 1954 from Sugar Creek, a small tributary of the Portage River, which enters western Lake Erie at Port Clinton, Ohio. The stream temperatures at the times of collection varied between 22 and 26° C. The animals were maintained at Stone Institute in shallow tanks of running lake water, the temperature of which ranged betwen 22 and 26° (usually between 23 and 24°), and the pH between 8.4 and 8.7 (usually between 8.5 and 8.6). The crayfish seemed to remain in good health under these conditions—they fed eagerly on mussel, fish, bread and lettuce, many molted, the mortality was low, and those not removed for use in the experiments were alert and lively after about six weeks.

Aside from several dozen adult crayfish used only to test the effects of size on heat-tolerance, the seven or eight hundred specimens used in the course of the study ranged between 17 and 42 mm. in total length (from the tip of the rostrum to the tip of the telson when straightened out), most of them being between 25 and 35 mm. They were sexually immature, and presumably in their first summer of life (Van Deventer, 1937; Tack, 1941). Groups of from 25 to 100 were taken at random from the stock tanks and maintained in well-aerated glass aquaria at acclimatization temperatures of 30 ± 0.1, 12 ± 0.5 and 4 ± 0.5°. Those held at 30° were warmed to this temperature from that of the stock tank over a period of several hours. Those in the low-temperature baths were transferred directly from the stock tank, it having been found unnecessary to cool them slowly. Care was taken that no metal came into contact with the water in the aquaria. The animals at 30 and 12° were fed bread and lettuce; those at 4° did not feed. About a fourth of the water in each aquarium was replaced every day or so with fresh lake water, previously brought to the appropriate acclimatization temperature and aerated. The crayfish remaining in the stock tanks were used as controls.

The heat-tolerance of control and experimental animals was studied by testing their survival for 12 hours or longer at 33, 34, 35, 36 and 37°, it having been ascertained in preliminary tests that this range of temperatures spanned the 12-hour median heat-tolerance limits (Doudoroff, 1942; Brett, 1944) of the several acclimatized groups. The test temperatures were maintained to within 0.1°. The animals were tested singly in 10-oz. wide-mouth bottles or in groups of 5 to 10 in one-liter Erlenmeyer flasks, these vessels being held at the test temperatures in constant temperature baths. Each bath held a number of such vessels, so that it was possible to test as many as 40 crayfish simultaneously at one temperature. In preparation for a test, aerated fresh lake water was brought to the temperature of the acclimatization bath from which the animals were to be taken. One hundred ml. of this water were then poured into each bottle, or one liter into each flask, so that at least 100 ml. of water were provided for each animal being tested. The crayfish were then transferred to the vessels and warmed gradually to the test temperature, the test period being started as soon as this temperature had been reached. Aeration sufficient to maintain an oxygen concentration of at least 80% air-saturation was continued throughout the warming and test periods.

The warming period usually lasted for from 15 to 60 minutes, according to the difference between the acclimatization and test temperatures. This procedure was chosen in preference to that of transferring the crayfish abruptly from the acclimatization temperature to the test temperature because the property under consideration was the ability to survive for 12 hours or longer at the test temperature, rather than that of being able to withstand the shock of a sudden plunge from one temperature extreme to another. A sudden plunge into warm water was found to constitute so massive a shock, especially when the difference between the two temperatures spanned some 25 degrees or more, that the distress of the animals was intense, sometimes enough to cause them to throw off one or both chelae. It is possible, of course, that the period of warming allowed them to gain somewhat in heat-tolerance over that possessed at the acclimatization temperature, but such a gain in tolerance, if it occurred at all, was not great enough to obscure the results. In this connection Brett (1941) has reported that raising the temperature gradually from acclimatization to test temperatures over a period of 15 minutes had no effect on the lethal temperatures of speckled trout.

The condition of each crayfish was observed as it reached the test temperature, and from time to time in the course of the ensuing test period. When no movement of the appendages could be detected, or elicited by prodding with a glass rod, the crayfish was considered to be dead. Death was confirmed in each case at room temperature after the test, and at this time the length of each animal was measured, the sex determined, and gastroliths sought. Each gastrolith found was measured along its greatest diameter, and its thickness noted, the size serving as an index to the stage of the molt cycle (Scudamore, 1947). As the responses of the crayfish tested in 1954 were practically the same as those of the 1953 collection, the data of the two years have been combined.

Results and Interpretation

1. *Heat-tolerance of control animals*

Crayfish that had been maintained in the stock tanks for from one to five weeks at temperatures between 22 and 26° survived for not more than 6 hours at 37°, for more than 12 but usually not more than 24 hours at 36°, for at least 10 days at 35°, and for at least 24 days at 34°. Their survival of 12-hour and 24-hour exposures to the three higher temperatures is shown in Table I. Evidently the 12-hour median tolerance limit for these animals was above 36°, but below 37°. When the per cent surviving at each temperature is plotted against the test temperature (Figure 1), the 12-hour median heat-tolerance limit is seen to have been in the vicinity of 36.4°. That for 24 hours was 35.6°.

Table I

Survival of crayfish taken from temperatures between 22 and 26° and tested at 37, 36 and 35°

Test temperature °C.	Number of tests	Number of crayfish	Crayfish surviving			
			for 12 hours		for 24 hours	
			Number	Per cent	Number	Per cent
37	9	76	0	0	—	—
36	15	109	86	78.9	19	17.4
35	12	78	78	100	73	93.6

Their survival for long periods at 34 and 35° confirms and extends Bovbjerg's (1952) observations. In view of the proximity of these two temperatures to the 24-hour median tolerance limit of the controls, it is of interest that the crayfish molted and fed at 34 and 35°. The small difference between survival- and death-temperatures is quite striking, although not peculiar to the crayfish. It has been observed before in experiments with the sand crab (Edwards and Irving, 1943) and with the greenfish, *Girella nigricans* (Doudoroff, 1942).

2. *Loss of heat tolerance*

When transferred from the stock tanks to an acclimatization bath at 4 ± 0.5°, the crayfish lost the heat-tolerance characteristic of the control group, the loss becoming greater the longer the exposure to low temperature. The 12-hour median heat-tolerance limit fell from 36.4 to 35.3° by the end of the fourth day, to 34.8° by the end of the eighth day, to 34.1° by the end of the twelfth day, and to 33.5° by the end of the sixteenth day in the cold, as shown in Figure 2. Apparently the loss of tolerance did not begin immediately upon exposure to the cold, however, the tolerance after two and a half days being about the same as the original value. The fact that the minnow, *Pimephales promelas,* shows a similar latent period in the loss of heat-tolerance (Brett, 1944) suggests that this may be a rather general characteristic of animals. The apparent slight increase in tolerance after one day in the cold may have been due to chance, the number of crayfish tested at this point being rather small, but it seems worth noting in this

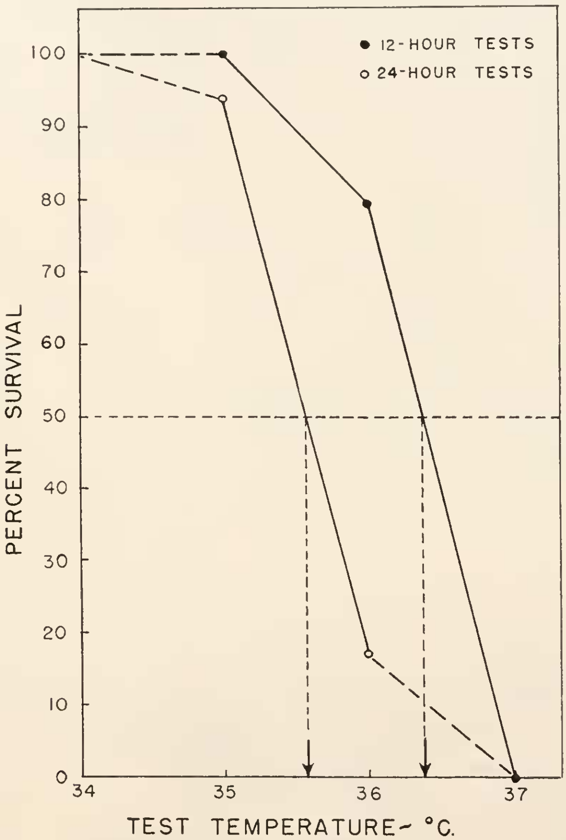

FIGURE 1. Survival at 35, 36 and 37° of crayfish acclimatized to temperatures between 22 and 26° C. The arrows mark the 12- and 24-hour median heat-tolerance limits.

connection that Sumner and Doudoroff (1938) and Doudoroff (1942) have made similar observations on two species of fish. Presumably the crayfish would have continued to lose heat-tolerance, had they been left longer at 4°.

Stock crayfish transferred to 12 ± 0.5° lost their original heat-tolerance in a similar manner, but more slowly. As shown in Figure 2, the 12-hour median tolerance limit had fallen to 35.6° by the end of five and a half days, and to 35.1° by the end of the second week.

3. Gain of heat-tolerance

The heat-tolerance lost during exposure to low temperature was regained quite rapidly when the crayfish were removed from the cold bath. Twenty specimens that had been held at 4 ± 0.5° for thirteen days, long enough for them to have lost their original heat-tolerance by more than two degrees and to have reached a median tolerance limit of about 34° (Fig. 2), were warmed to room temperature (23.5 to 24°) in the course of about two hours and maintained at this temperature for 22 hours. When they were then tested at 36°, 90% lived for 12 hours and 40% survived 24 hours at the test temperature. Evidently their heat-tolerance was at least as great as that of the control animals from the stock tanks, the

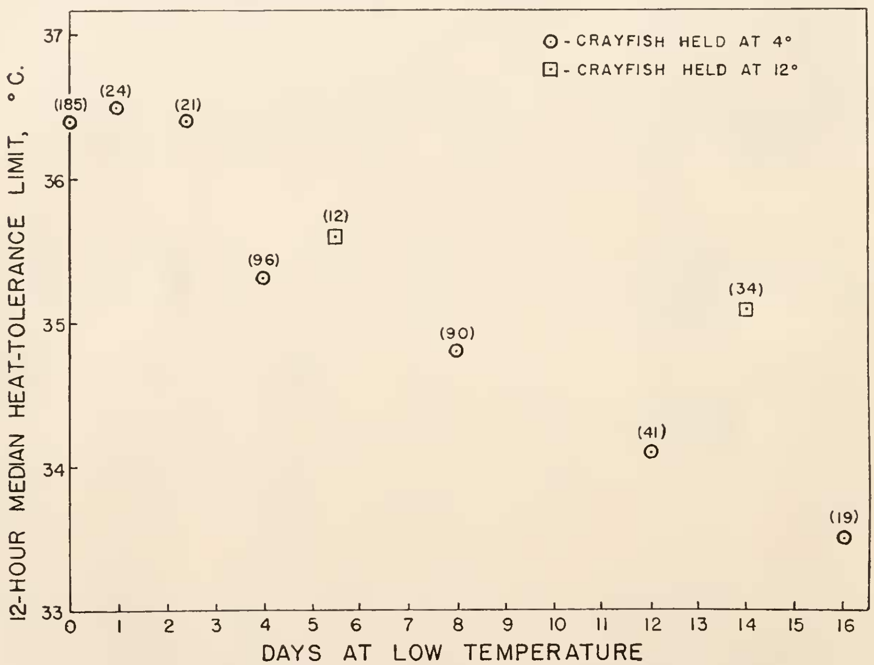

FIGURE 2. Loss of heat-tolerance at 4 ± 0.5 and 12 ± 0.5° C. Numbers of test animals shown in parentheses.

survival of the latter at 36° averaging 78.9% for 12 hours and 17.4% for 24 hours (Table I). Results consistent with these were obtained with six crayfish tested at 35° after one day, and with nine tested at 36° after two days, at room temperature, five of the six and seven of the nine surviving the 12-hour test periods. As in fish (Doudoroff, 1942; Brett, 1944, 1946), the rate of gain of heat-tolerance by the crayfish seems to be considerably higher than the rate of loss, the tolerance lost in about ten days at 4° (after the first two and a half days had elapsed) being recovered in not more than one day.

Upon acclimatization to a higher temperature, the crayfish gained a heat-tolerance somewhat greater than that characteristic of acclimatization to 22–26°.

The 12-hour and 24-hour median heat-tolerance limits of a group of crayfish maintained in an aquarium at 30 ± 0.1° were compared with those of a control group maintained under the same conditions in another aquarium, but at room temperature (between 22 and 25°). Both groups were tested over a three-week period, 20 of each group being tested at the end of each of the first two weeks, and 10 of each group at the end of the third week. The 12-hour median tolerance limit of the 30° animals was 36.6° by the end of the first week and remained at this level for the next two weeks. The corresponding limits for the controls were 36.2, 36.4 and 36.5° after the first, second and third weeks, respectively. The 24-hour limit also was 36.6° at the end of the first week and, like that for 12 hours, did not change from this value during the second and third weeks. The corresponding weekly 24-hour tolerance limits for the controls were 35.5, 35.6 and 35.5°. The slight increase in the 12-hour median tolerance limit is not in itself convincing, but the increase by about one degree in the 24-hour tolerance limit represents a clear-cut gain. The reality of this gain is indicated by the fact that the three tests were consistent, although conducted a week apart. Furthermore, five of the animals from the 30° aquarium lived for more than 12 hours at 37°, one of them, in fact, surviving a 24-hour test at this temperature. It will be recalled that none of the controls taken from temperatures between 22 and 26° lived for more than 6 hours at 37°. It is of interest that continued exposure to 30° did not cause an increase in tolerance over that shown by the end of the first week, and that the limit for 12 hours reached a value no higher than that for 24 hours. These observations suggest the possibility that 36.6° was not far from the maximal heat-tolerance limit attainable by the population from which the collections were made. In view of the high rate of gain of heat-tolerance described earlier, it seems not improbable that acclimatization to 30° was completed in much less than a week.

4. Heat-tolerance in relation to sex, size and the molt cycle

Although all crayfish used in these tests were drawn from the same population and maintained under the same conditions, they were of course not all alike. Males and females were about equally represented, the lengths ranged from 17 to 42 mm., and the animals were at different stages of their molt cycles. About 3% of the specimens had gastroliths large enough to indicate that they were at the molting stage; the rest had smaller gastroliths (about 22%) or none that could be seen macroscopically (75%). As the crayfish were distributed randomly among the tests, it seems unlikely that the results would have been affected by these variables, even if there had existed a relationship between heat-tolerance and sex, size, or the stage of the molt cycle. As a matter of fact, the evidence at hand indicates that no such relationship existed.

Because of the random distribution, males and females, large and small specimens, and representatives of different stages in the molt cycle could be found in each series of tests. The effects of each of these variables could be investigated, therefore, by comparing the animals that survived with those that died at each test temperature. In this manner it was found that as in the sand crab (Edwards and Irving, 1943) and the amphipod, *Hyalella azteca* (Bovee, 1949), sex did not affect the heat-tolerance of the crayfish. It must be borne in mind, of course, that the specimens were immature. No relationship between size and tolerance could be detected, and the absence of such a relationship is indicated further by the fact that 30 adult specimens, of body lengths between 56 and 82 mm. and with the same history as the control animals in the stock tanks, were neither more nor less heat tolerant than the controls. No inconsistency is seen between these results and reports that larger specimens of the sand crab (Edwards and Irving, 1943) and *Hyalella azteca* (Bovee, 1949) were somewhat more heat resistant than small ones, the methods used in the present study being so different from the others that no direct comparison can be made. Using methods essentially the same as those described in this paper, Doudoroff (1942) and Hart (1947) found no correlation between size and survival time of fish at extreme temperatures. Finally, the stage of the molt cycle evidently had no effect upon heat-tolerance, despite the changes associated with molting and the stress thought to be placed upon the crayfish by the process (Scudamore, 1947). Whether the gastroliths were absent, small, or so large as to indicate that the animals were in molting condition, there was no evidence that their tolerance had been affected. Furthermore, crayfish were found to molt successfully in the acclimatization baths at 12 and 30°, in the course of tests at temperatures as high as 36°, and at room temperature after the period of exposure to the test temperature. They did not molt at 4°.

Discussion

The literature on temperature relations of fish has made it clear that heat-tolerance is so dependent upon acclimatization temperature that expressions of either lethal temperatures or median tolerance limits are incomplete unless accompanied by information on the animal's thermal history. According to the results of the present tests, as well as those described by Bovbjerg (1952), the same can be said of the crayfish. As a consequence, an attempt to compare the heat-tolerance of the crayfish with those of other aquatic animals is hampered by the fact that in most cases the acclimatization temperatures have been different from those used in this study. Such a comparison is limited further by differences among the criteria used in the measurement of heat-tolerance. Whereas 12- and 24-hour median tolerance limits have been sought here, largely so that the results could be related to the more highly developed literature on the temperature relations of fish, most of the data on aquatic arthropods have been expressed in terms either of the death temperatures of animals subjected to ever-increasing temperatures or of the time required for them to die when transferred to constant test temperatures.

Although one gains the general impression that the crayfish is among the more heat tolerant of the aquatic arthropods that have been investigated, direct comparisons are attempted here with but five species (Table II), these having been selected because their acclimatization temperatures were reasonably close to that of the crayfish from the stock tanks. The median tolerance limit given in Table II for *Orconectes rusticus* is that for the 12-hour test period. The figure for *Hyalella* is the temperature at which about half the specimens survived for nearly 11 hours, so that it approximates the 12-hour median tolerance limit. It seems unlikely that increasing the acclimatization temperature by two or three degrees would have increased the median tolerance limit to that of the crayfish. In fish, at any rate, the acclimatization temperature must be increased by several degrees to bring about an increase of one degree in the median tolerance limit (Fry, Brett and Clawson, 1942; Brett, 1944; Hart, 1947). A temperature of 35° is almost certainly lower than the 12-hour median heat-tolerance limit of Bovbjerg's (1952) acclimatized crayfish, inasmuch as they lived for days between 34 and 35°. On the other hand, 37° is probably somewhat above that of the sand crab, all specimens tested by Edwards and Irving (1943) having died during or shortly after a period of four hours at this temperature. The figures shown for the lobster larvae are the temperatures at which the hearts stopped beating as the animals were warmed at a rate approximating 0.4° a minute; the 12-hour median tolerance limits must have been considerably lower. Although conclusions can be no more than tentative, the sand crab and the three species of crayfish appear to have similar heat-tolerance limits, these being greater by several degrees than those of the lobster larvae and *Hyalella azteca*.

The methods and criteria being more nearly alike, the temperature relations of the crayfish can be compared with those of fish with more confidence. Evidently the crayfish has a heat-tolerance of the same order as that of the more tolerant of

Table II

Estimated heat-tolerance limits of several aquatic arthropods related to the 12-hour median heat-tolerance limit of Orconectes rusticus acclimatized to 22–26°

Animal	Acclimatization temperature, °C.	Heat-tolerance limit, °C.	Author
Orconectes rusticus	22–26	36.4	—
O. propinquus	18–28	more than 35	Bovbjerg, 1952
Cambarus fodiens	18–28	more than 35	Bovbjerg, 1952
Lobster, stage 4	25	less than 34.2	Huntsman, 1924
Lobster, stage 5	20	less than 35.4	Huntsman, 1924
Emerita talpoida	20	less than 37	Edwards and Irving, 1943
Hyalella azteca	20	33	Bovee, 1949

the fishes. Of the several dozen species of fresh water fish for which comparable data are available (Fry, Brett and Clawson, 1942; Brett, 1944; Hart, 1947; Black, 1953), the acclimatization temperatures and criteria of tolerance being essentially the same as those used in this study, the brown bullhead (*Ameiurus nebulosus*) and the goldfish (*Carassius auratus*) are among the most tolerant of high temperatures. That crayfish are at least as tolerant as the bullhead is indicated by the fact that bullheads acclimatized to 26° had a lethal temperature (the equivalent of the 12-hour median heat-tolerance limit used here) of 35.3° (Brett, 1944), whereas the corresponding limit for crayfish of similar acclimatization was 36.4°. Apparently the crayfish does not exceed the bullhead in its potential range of tolerance, however, for the 12-hour median tolerance limit of crayfish acclimatized to 30° was about the same (36.6°) as that indicated for the bullhead by Brett's (1944) data. Like the bullhead, the crayfish appears to be less heat tolerant than the goldfish, specimens of which had a 12-hour median heat-tolerance limit somewhat greater than 37° when acclimatized to 28°, according to figures given by Brett (1946). The rather marked similarities between crayfish and fish with respect to loss and gain of heat-tolerance, as described in a preceding section, suggest that the basic principles of temperature acclimatization are much alike in the two groups, if not identical.

Summary

1. Crayfish (*Orconectes rusticus*) taken from environmental temperatures between 22 and 26° lived for not more than six hours at 37° and for at least ten days at 35°. Their 12-hour and 24-hour median heat-tolerance limits were 36.4 and 35.6°, respectively.

2. Upon being transferred to about 4° and maintained at this temperature, their heat-tolerance, as measured by the 12-hour median tolerance limit, fell to 35.3° after four days, to 34.8° after eight days, to 34.1° after twelve days, and to 33.5° after sixteen days. Heat-tolerance was lost in a similar manner at about 12°, but more slowly.

3. The heat-tolerance lost after thirteen days of exposure to 4° was regained within one day after they had been returned to the original environmental temperature, the rate of gain of heat-tolerance being much higher than the rate of loss.

4. When tested after one week at 30°, the 24-hour median heat-tolerance limit had risen to 36.6° from the initial value of 35.6°. Two more weeks at 30° did not bring about a further increase in this tolerance limit, however.

5. Apparently the heat-tolerance of these crayfish was not affected by sex, size, or the stage of the molt cycle.

6. The results indicate that crayfish are among the more heat tolerant of the aquatic arthropods and fish for which appropriate data are available.

7. The similarities between the temperature relations of crayfish and fish suggest that the basic principles are alike in the two groups.

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